

FIG. 1

sequence_AA_2D12.5_variable domains.txt

```
>2D12.5VL_MOUSE
(1) QAVWTQESALTTSPGETVTLTCSRSTGAVTTSNYANWQEKPDHLFTGLIGGNNRPPGVPARFSGSLIGDKAALTIAGTQTED
    EAIYFCALWYSNHWVFEGGGTRLTVLG

(2) CDR1 - RSSTGAVTTSNYAN
(3) CDR2 - GNNNRPP
(4) CDR3 - ALWYSNHWV

>2D12.5VH_MOUSE
(5) QVKLQESGPGLVQPSQSLSTCTVSGFSLTDYGVHWRQSPGKGLEWLGVWSGGGTAYTAAFISRLNIY
    KDNSKNQVFFEMNSLQANDTAMYYCARRGSPYNYFDVWGQGTTVTVSS

(6) CDR1 - DYGVH
(7) CDR2 - VIWSGGGTAYTAAFIS
(8) CDR3 - RGSYPYNYFDV
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FIG. 2

Translation of 2D12.5 VH variable genes

		10	20	30	40	50
(9) 2d12.5 VH native hybridoma	1	VKLQESG	GLVQ	PSQSL	SITCTVSGFSLTDYGVHWVRQSPGKGLEWLGVI	50
(10) 2d12.5 VH native cloned	1	50
(11) 2d12.5 VH N87D_cloned	1	50
(12) 2d12.5 VH N87D_G53C_cloned	1	50
(13) 2d12.5 VH N87D_G54C_cloned	1	50
(14) 2d12.5 VH N87D_G55C_cloned	1	50
		60	70	80	90	100
2d12.5 VH native hybridoma	51	WSGGGTAYTAA	FISRLNIYKD	NSKNQVFFEM	NSLQANDTAMY	YCARRGSY 100
2d12.5 VH native cloned	51	100
2d12.5 VH N87D_cloned	51	D.....	100
2d12.5 VH N87D_G53C_cloned	51	..C.....	D.....	100
2d12.5 VH N87D_G54C_cloned	51	..C.....	D.....	100
2d12.5 VH N87D_G55C_cloned	51	..C.....	D.....	100
		110				
2d12.5 VH native hybridoma	101	PYNYFDVWG	QGTTVT	VSS		118
2d12.5 VH native cloned	101	A		118
2d12.5 VH N87D_cloned	101	A		118
2d12.5 VH N87D_G53C_cloned	101	A		118
2d12.5 VH N87D_G54C_cloned	101	A		118
2d12.5 VH N87D_G55C_cloned	101	A		118

FIG. 3A

2D12.5 VH variable genes

			10	20	30	40	50	
(15) 2d12.5 VH nativ hybridoma	1	GTGAAGCTGCAGGAGTCAGGACCTGGCCTAGTGCAGCCCTCACAGAGCCT	50					
(16) 2d12.5 VH native cloned	1T.....	50					
(17) 2d12.5 VH N87D_cloned	1T.....	50					
(18) 2d12.5 VH N87D_G53C_cloned	1T.....	50					
(19) 2d12.5 VH N87D_G54C_cloned	1T..G.....	50					
(20) 2d12.5 VH N87D_G55C_cloned	1T.....	50					
			60	70	80	90	100	
2d12.5 VH native hybridoma	51	GTCCATCACCTGCACGGTCTCTGGTTTCTCATTAACTGACTATGGTGTAC	100					
2d12.5 VH native cloned	51	100					
2d12.5 VH N87D_cloned	51	100					
2d12.5 VH N87D_G53C_cloned	51	100					
2d12.5 VH N87D_G54C_cloned	51	100					
2d12.5 VH N87D_G55C_cloned	51	100					
			110	120	130	140	150	
2d12.5 VH native hybridoma	101	ACTGGGTTCCGCGAGTCTCCAGGAAAGGGTCTGGAATGGCTGGGAGTGATA	150					
2d12.5 VH native cloned	101	150					
2d12.5 VH N87D_cloned	101	150					
2d12.5 VH N87D_G53C_cloned	101	150					
2d12.5 VH N87D_G54C_cloned	101	150					
2d12.5 VH N87D_G55C_cloned	101	150					
			160	170	180	190	200	
2d12.5 VH native hybridoma	151	TGGAGTGGTGGAGGCACGGCCTATACTGCGGCGTTTCATATCCAGACTGAA	200					
2d12.5 VH native cloned	151	200					
2d12.5 VH N87D_cloned	151	200					
2d12.5 VH N87D_G53C_cloned	151T.....	200					
2d12.5 VH N87D_G54C_cloned	151T..T.....	200					
2d12.5 VH N87D_G55C_cloned	151T.....	200					
			210	220	230	240	250	
2d12.5 VH native hybridoma	201	CATCTACAAGGACAATTCCAAGAACCAAGTTTTCTTTGAAATGAACAGTC	250					
2d12.5 VH native cloned	201	250					
2d12.5 VH N87D_cloned	201	250					
2d12.5 VH N87D_G53C_cloned	201	250					
2d12.5 VH N87D_G54C_cloned	201	250					
2d12.5 VH N87D_G55C_cloned	201	250					
			260	270	280	290	300	
2d12.5 VH native hybridoma	251	TGCAAGCTAATGACACAGCCATGTATTACTGTGCCAGAAGGGGTAGCTAC	300					
2d12.5 VH native cloned	251	300					
2d12.5 VH N87D_cloned	251G.....	300					
2d12.5 VH N87D_G53C_cloned	251G.....	300					
2d12.5 VH N87D_G54C_cloned	251G.....	300					
2d12.5 VH N87D_G55C_cloned	251G.....	300					
			310	320	330	340	350	
2d12.5 VH native hybridoma	301	CCTTACAATACTCTCGATGTCTGGGGCCAAGGGACACAGTCACCGTCTC	350					
2d12.5 VH native cloned	301G.....	350					
2d12.5 VH N87D_cloned	301G.....	350					
2d12.5 VH N87D_G53C_cloned	301G.....	350					
2d12.5 VH N87D_G54C_cloned	301G.....	350					
2d12.5 VH N87D_G55C_cloned	301G.....	350					

2D12.5 VH variable genes

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.....
2d12.5 VH native hybridoma 351 CTCA 354
2d12.5 VH native cloned    351 .G.. 354
2d12.5 VH N87D_cloned      351 .G.. 354
2d12.5 VH N87D_G53C_cloned 351 .G.. 354
2d12.5 VH N87D_G54C_cloned 351 .G.. 354
2d12.5 VH N87D_G55C_cloned 351 .G.. 354
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FIG. 4

Translation of 2D12.5 VL genes

			10	20	30	40	50	
(21) 2d12.5 VL native hybridoma	1	AVVTQESALTTSPGETVTLTCRSSTGAVTTTSNYANWVQEKPDHLFTGLIG	50				
(22) 2d12.5 VL native cloned	1		50				
(23) 2d12.5 VL N53C_cloned	1		50				
			60	70	80	90	100	
2d12.5 VL native hybridoma	51	GNNNRPPGVPARFSGSLIGDKAALTIAGTQTEDEAIYFCALWYSNHWVFG	100				
2d12.5 VL native cloned	51		100				
2d12.5 VL N53C_cloned	51	.C.....		100				
							
2d12.5 VL native hybridoma	101	GGTRLTVLG	109					
2d12.5 VL native cloned	101	...K....S	109					
2d12.5 VL N53C_cloned	101	...K....S	109					

FIG. 5

			10	20	30	40	50	
							
(24)	2d12.5 VL native hybridoma	1	GCTGTTGTGACTCAGGAATCTGCACTCACCACATCACCTGGTGAAACAGT					50
(25)	2d12.5 VL native cloned	1					50
(26)	2d12.5 VL N53C cloned	1					50
			60	70	80	90	100	
							
	2d12.5 VL native hybridoma	51	CACACTCACTTGTCGCTCAAGTACTGGGGCTGTTACGACTAGTAACTATG					100
	2d12.5 VL native cloned	51					100
	2d12.5 VL N53C cloned	51					100
			110	120	130	140	150	
							
	2d12.5 VL native hybridoma	101	CCAACTGGGTCCAAGAGAAAACAGATCATTATTCACTGGTCTAATAGGT					150
	2d12.5 VL native cloned	101					150
	2d12.5 VL N53C cloned	101					150
			160	170	180	190	200	
							
	2d12.5 VL native hybridoma	151	GGTAATAATAACCGACCTCCAGGTGTTCTCCTGCCAGATTCTCAGGCTCCCT					200
	2d12.5 VL native cloned	151					200
	2d12.5 VL N53C cloned	151	...TG.....					200
			210	220	230	240	250	
							
	2d12.5 VL native hybridoma	201	GATTGGAGACAAGGCTGCCCTCACCATCGCAGGGACACAGACTGAGGATG					250
	2d12.5 VL native cloned	201					250
	2d12.5 VL N53C cloned	201					250
			260	270	280	290	300	
							
	2d12.5 VL native hybridoma	251	AGGCAATATATTTCTGTGCTCTATGGTACAGCAACCATTGGGTGTTCCGGT					300
	2d12.5 VL native cloned	251					300
	2d12.5 VL N53C cloned	251					300
			310	320				
							
	2d12.5 VL native hybridoma	301	GGAGGAACCAGACTGACTGTCCTAGGC					327
	2d12.5 VL native cloned	301	..G.....A.....A..					327
	2d12.5 VL N53C cloned	301	..G.....A.....A..					327

$$S_{\text{max}} = \frac{1}{2} \left(\frac{1}{\lambda_1} + \frac{1}{\lambda_2} \right) \left(\frac{1}{\lambda_1} + \frac{1}{\lambda_2} \right) = \frac{1}{2} \left(\frac{1}{\lambda_1} + \frac{1}{\lambda_2} \right)^2$$

Translation of Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

(27)	2dVL-TTCL native_cloned	1	RSVVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGL	50
(28)	2dVL-TTCL N53C_cloned	1	50
(29)	2d12.5 VL native hybridoma	1	48
(30)	TTCL template for gene assembl	1	-----	1
			60 70 80 90 100	
	2dVL-TTCL native_cloned	51	IGGNNRRPPGVPARFSGSLIGDKAALTIAGTQTEDEAIYFCALWYSNHWV	101
	2dVL-TTCL N53C_cloned	51	...C.....	10
	2d12.5 VL native hybridoma	49	49
	TTCL t mplate for gene assembl	1	-----	1
			110 120 130 140 150	
	2dVL-TTCL native_cloned	101	FGGGTKLTVLSRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV	151
	2dVL-TTCL N53C_cloned	101	151
	2d12.5 VL native hybridoma	99R....G	10
	TTCL template for gene assembl	1	-----	39
			160 170 180 190 200	
	2dVL-TTCL native_cloned	151	QWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEEKHKVYACEV	201
	2dVL-TTCL N53C_cloned	151	201
	2d12.5 VL native hybridoma			
	TTCL template for gene assembl	40	80
			210 220	
	2dVL-TTCL native_cloned	201	THQGLSLPVTKSFNRGEC*F*	221
	2dVL-TTCL N53C_cloned	201*.*	221
	2d12.5 VL native hybridoma			
	TTCL template for gene assembl	90	107

FIG. 7A

Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

			10	20	30	40	50	
(31)	2dVL-TTCL native_cloned	1	AGATCTGCTGTTGTGACTCAGGAATCTGCACTCACCACATCACCTGGTGA	50				
(32)	2dVL-TTCL N53C_clon d	1	50				
(33)	2d12.5 VL native hybridoma	1	-----	44				
(34)	TTCL template for gene assem	1	-----	1				
			60	70	80	90	100	
	2dVL-TTCL native_cloned	51	AACAGTCACACTCACTTGTCGCTCAAGTACTGGGGCTGTTACGACTAGTA	100				
	2dVL-TTCL N53C_cloned	51	100				
	2d12.5 VL native hybridoma	45	94				
	TTCL template for gene assem	1	-----	1				
			110	120	130	140	150	
	2dVL-TTCL native_cloned	101	ACTATGCCAACTGGGTCCAAGAGAAACCAGATCATTATTACTGGTCTA	150				
	2dVL-TTCL N53C_cloned	101	150				
	2d12.5 VL native hybridoma	95	14				
	TTCL template for gene assem	1	-----	1				
			160	170	180	190	200	
	2dVL-TTCL native_cloned	151	ATAGGTGGTAATAATAACCGACCTCCAGGTGTTCTGCCAGATTCTCAGG	20				
	2dVL-TTCL N53C_cloned	151TG.....	20				
	2d12.5 VL native hybridoma	145	19				
	TTCL template for gene assem	1	-----	1				
			210	220	230	240	250	
	2dVL-TTCL native_cloned	201	CTCCCTGATTGGAGACAAGGCTGCCCTCACCATCGCAGGGACACAGACTG	25				
	2dVL-TTCL N53C_cloned	201	25				
	2d12.5 VL native hybridoma	195	24				
	TTCL template for gene assem	1	-----	1				
			260	270	280	290	300	
	2dVL-TTCL native_cloned	251	AGGATGAGGCAATATATTTCTGTGCTCTATGGTACAGCAACCATTGGGTG	30				
	2dVL-TTCL N53C_cloned	251	30				
	2d12.5 VL native hybridoma	245	28				
	TTCL template for gene assem	1	-----	1				
			310	320	330	340	350	
	2dVL-TTCL native_cloned	301	TTCGGTGGGGGAACCAAACCTGACTGTCCTAAGCCGAACCTGTGGCTGCACC	3				
	2dVL-TTCL N53C_cloned	301	3				
	2d12.5 VL native hybridoma	295A.....G.....G..	3				
	TTCL template for gene assem	1	-----	1				
			360	370	380	390	400	
	2dVL-TTCL native_cloned	351	ATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTG	4				
	2dVL-TTCL N53C_cloned	351	4				
	2d12.5 VL native hybridoma						
	TTCL template for gene assem	18					
			410	420	430	440	450	
	2dVL-TTCL native_clon d	401	CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTA					
	2dVL-TTCL N53C_clon d	401					
	2d12.5 VL native hybridoma						
	TTCL template for gene assem	68					

FIG. 7B

Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

		460	470	480	490	500
					
2dVL-TTCL native_cloned	451	CAGTGGAAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGT	500			
2dVL-TTCL N53C_cloned	451	500			
2d12.5 VL native hybridoma						
TTCL template for gene assem	118	161			
		510	520	530	540	550
					
2dVL-TTCL native_cloned	501	CACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGA	550			
2dVL-TTCL N53C_cloned	501	550			
2d12.5 VL native hybridoma						
TTCL template for gene assem	168	211			
		560	570	580	590	600
					
2dVL-TTCL native_cloned	551	CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTC	600			
2dVL-TTCL N53C_cloned	551	600			
2d12.5 VL native hybridoma						
TTCL template for gene assem	218	261			
		610	620	630	640	650
					
2dVL-TTCL native_cloned	601	ACCCATCAGGGCCTGAGCTTGCCCGTCACAAAGAGCTTCAACAGGGGAGA	650			
2dVL-TTCL N53C_cloned	601T.....	650			
2d12.5 VL native hybridoma						
TTCL template for gene assem	268	311			
		660				
					
2dVL-TTCL native_cloned	651	GTGTTAATTCTAGA	664			
2dVL-TTCL N53C_cloned	651	664			
2d12.5 VL native hybridoma						
TTCL template for gene assem	318	322			

FIG. 8

Translation of Mous 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

			10	20	30	40	50	
(35) 2dVH-TTCH_native cloned	1	RSVKLQESGPG	GLVQPSQSL	SITCTVSGF	SLTDYGVH	WVRQSPG	KGLEWL	50
(36) 2dVH-TTCH_N87D_cloned	1	50
(37) 2dVH-TTCH_N87D_G53C_cloned	1	50
(38) 2dVH-TTCH_N87D_G54C_cloned	1	50
(39) 2dVH-TTCH_N87D_G55C_cloned	1	50
(40) 2dVH-TTCH expected sequence	1	50
(41) 2d12.5 VH native hybridoma	1	48
			60	70	80	90	100	
2dVH-TTCH_native cloned	51	VIWSGGGTAY	TAAAFISRL	NIYKDNSKN	QVFFEMNSL	QANDTAMY	YCARRG	100
2dVH-TTCH_N87D_cloned	51	D.....	100
2dVH-TTCH_N87D_G53C_cloned	51	C.....	D.....	100
2dVH-TTCH_N87D_G54C_cloned	51	C.....	D.....	100
2dVH-TTCH_N87D_G55C_cloned	51	C.....	D.....	100
2dVH-TTCH expected sequence	51	100
2d12.5 VH native hybridoma	49	98
			110	120	130	140	150	
2dVH-TTCH_native cloned	101	SYPYNYFDV	WGQGTTVT	VSAASTKG	PSVFPLA	SSKSTSGG	TAAALGCL	150
2dVH-TTCH_N87D_cloned	101	150
2dVH-TTCH_N87D_G53C_cloned	101	150
2dVH-TTCH_N87D_G54C_cloned	101	150
2dVH-TTCH_N87D_G55C_cloned	101	150
2dVH-TTCH expected sequence	101	150
2d12.5 VH native hybridoma	99	S.....	118
			160	170	180	190	200	
2dVH-TTCH_native cloned	151	DYFPEPVTV	SWNSGALT	SGVHTFPA	VLQSSGLY	SLSSVVTV	FSSSLGTQ	200
2dVH-TTCH_N87D_cloned	151	200
2dVH-TTCH_N87D_G53C_cloned	151	200
2dVH-TTCH_N87D_G54C_cloned	151	200
2dVH-TTCH_N87D_G55C_cloned	151	200
2dVH-TTCH expected sequence	151	200
2d12.5 VH native hybridoma		
			210	220				
2dVH-TTCH_native cloned	201	YICNVNHKPS	NTKVDDKA	EPKSCDKSR				227
2dVH-TTCH_N87D_cloned	201				227
2dVH-TTCH_N87D_G53C_cloned	201				227
2dVH-TTCH_N87D_G54C_cloned	201				227
2dVH-TTCH_N87D_G55C_cloned	201				227
2dVH-TTCH expected sequence	201				227
2d12.5 VH native hybridoma					

FIG. 9A

Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

		10	20	30	40	50	
(42) 2dVH-TTCH_native cloned	1	AGATCTGTGAAGCTGCAGGAGTCTGGACCTGGCCTAGTGCAGCCCTCACA	50				
(43) 2dVH-TTCH_N87D_cloned	1	50				
(44) 2dVH-TTCH_N87D_G53C_cloned	1	50				
(45) 2dVH-TTCH_N87D_G54C_cloned	1G.....	50				
(46) 2dVH-TTCH_N87D_G55C_cloned	1	50				
(47) 2dVH-TTCH expected sequence	1	50				
(48) 2d12.5 VH native hybridoma	1A.....	44				
		60	70	80	90	100	
2dVH-TTCH_native cloned	51	GAGCCTGTCCATCACCTGCACGGTCTCTGGTTTCTCATTAACTGACTATG	100				
2dVH-TTCH_N87D_cloned	51	100				
2dVH-TTCH_N87D_G53C_cloned	51	100				
2dVH-TTCH_N87D_G54C_cloned	51	100				
2dVH-TTCH_N87D_G55C_cloned	51	100				
2dVH-TTCH expected sequence	51	100				
2d12.5 VH native hybridoma	45	94				
		110	120	130	140	150	
2dVH-TTCH_native cloned	101	GTGTACACTGGGTTCCGCCAGTCTCCAGGAAAGGGTCTGGAATGGCTGGGA	150				
2dVH-TTCH_N87D_cloned	101	150				
2dVH-TTCH_N87D_G53C_cloned	101	150				
2dVH-TTCH_N87D_G54C_cloned	101	150				
2dVH-TTCH_N87D_G55C_cloned	101	150				
2dVH-TTCH expected sequence	101	150				
2d12.5 VH native hybridoma	95	144				
		160	170	180	190	200	
2dVH-TTCH_native cloned	151	GTGATATGGAGTGGTGGAGGCACGGCCTATACTGCGGCGTTCATATCCAG	200				
2dVH-TTCH_N87D_cloned	151	200				
2dVH-TTCH_N87D_G53C_cloned	151T.....	200				
2dVH-TTCH_N87D_G54C_cloned	151T.T.....	200				
2dVH-TTCH_N87D_G55C_cloned	151T.....	200				
2dVH-TTCH expected sequence	151	200				
2d12.5 VH native hybridoma	145	194				
		210	220	230	240	250	
2dVH-TTCH_native cloned	201	ACTGAACATCTACAAGGACAATTCCAAGAACCAAGTTTCTTTGAAATGA	250				
2dVH-TTCH_N87D_cloned	201	250				
2dVH-TTCH_N87D_G53C_cloned	201	250				
2dVH-TTCH_N87D_G54C_cloned	201	250				
2dVH-TTCH_N87D_G55C_cloned	201	250				
2dVH-TTCH expected sequence	201	250				
2d12.5 VH native hybridoma	195	244				
		260	270	280	290	300	
2dVH-TTCH_native cloned	251	ACAGTCTGCAAGCTAATGACACAGCCATGTATTACTGTGCCAGAAGGGGT	300				
2dVH-TTCH_N87D_cloned	251G.....	300				
2dVH-TTCH_N87D_G53C_cloned	251G.....	300				
2dVH-TTCH_N87D_G54C_cloned	251G.....	300				
2dVH-TTCH_N87D_G55C_cloned	251G.....	300				
2dVH-TTCH expected sequence	251	300				
2d12.5 VH native hybridoma	245	294				
		310	320	330	340	350	
						

FIG. 9B

Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

2dVH-TTCH_native clon d	301	AGCTACCCTTACAACCTACTTCGATGTCTGGGGCCAAGGGACCCACGGTCAC	350
2dVH-TTCH_N87D_cloned	301	350
2dVH-TTCH_N87D_G53C_cloned	301	350
2dVH-TTCH_N87D_G54C_cloned	301	350
2dVH-TTCH_N87D_G55C_cloned	301	350
2dVH-TTCH expected sequence	301	350
2d12.5 VH native hybridoma	295A.....	344

		360	370	380	390	400	
2dVH-TTCH_native cloned	351	CGTCTCCGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCT					400
2dVH-TTCH_N87D_cloned	351					400
2dVH-TTCH_N87D_G53C_cloned	351					400
2dVH-TTCH_N87D_G54C_cloned	351					400
2dVH-TTCH_N87D_G55C_cloned	351					400
2dVH-TTCH expected sequence	351					400
2d12.5 VH native hybridoma	345T..					354

		410	420	430	440	450	
2dVH-TTCH_native cloned	401	CCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG					450
2dVH-TTCH_N87D_cloned	401					450
2dVH-TTCH_N87D_G53C_cloned	401					450
2dVH-TTCH_N87D_G54C_cloned	401					450
2dVH-TTCH_N87D_G55C_cloned	401					450
2dVH-TTCH expected sequence	401					450
2d12.5 VH native hybridoma						

		460	470	480	490	500	
2dVH-TTCH_native cloned	451	GACTACTTCCCCGAACCGGTGACGGTGTCTTGGAACCTCAGGCGCCCTGAC					500
2dVH-TTCH_N87D_cloned	451					500
2dVH-TTCH_N87D_G53C_cloned	451					500
2dVH-TTCH_N87D_G54C_cloned	451					500
2dVH-TTCH_N87D_G55C_cloned	451					500
2dVH-TTCH expected sequence	451G.....					500
2d12.5 VH native hybridoma						

		510	520	530	540	550	
2dVH-TTCH_native cloned	501	CAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCTCAGGACTCTACT					550
2dVH-TTCH_N87D_cloned	501					550
2dVH-TTCH_N87D_G53C_cloned	501					550
2dVH-TTCH_N87D_G54C_cloned	501					550
2dVH-TTCH_N87D_G55C_cloned	501					550
2dVH-TTCH expected sequence	501					550
2d12.5 VH native hybridoma						

		560	570	580	590	600	
2dVH-TTCH_native cloned	551	CCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACC					600
2dVH-TTCH_N87D_cloned	551					600
2dVH-TTCH_N87D_G53C_cloned	551					600
2dVH-TTCH_N87D_G54C_cloned	551					600
2dVH-TTCH_N87D_G55C_cloned	551					600
2dVH-TTCH expected sequence	551					600
2d12.5 VH native hybridoma						

		610	620	630	640	650	
2dVH-TTCH_nativ cloned	601	TACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAA					650
2dVH-TTCH_N87D_cloned	601					650

FIG.9C

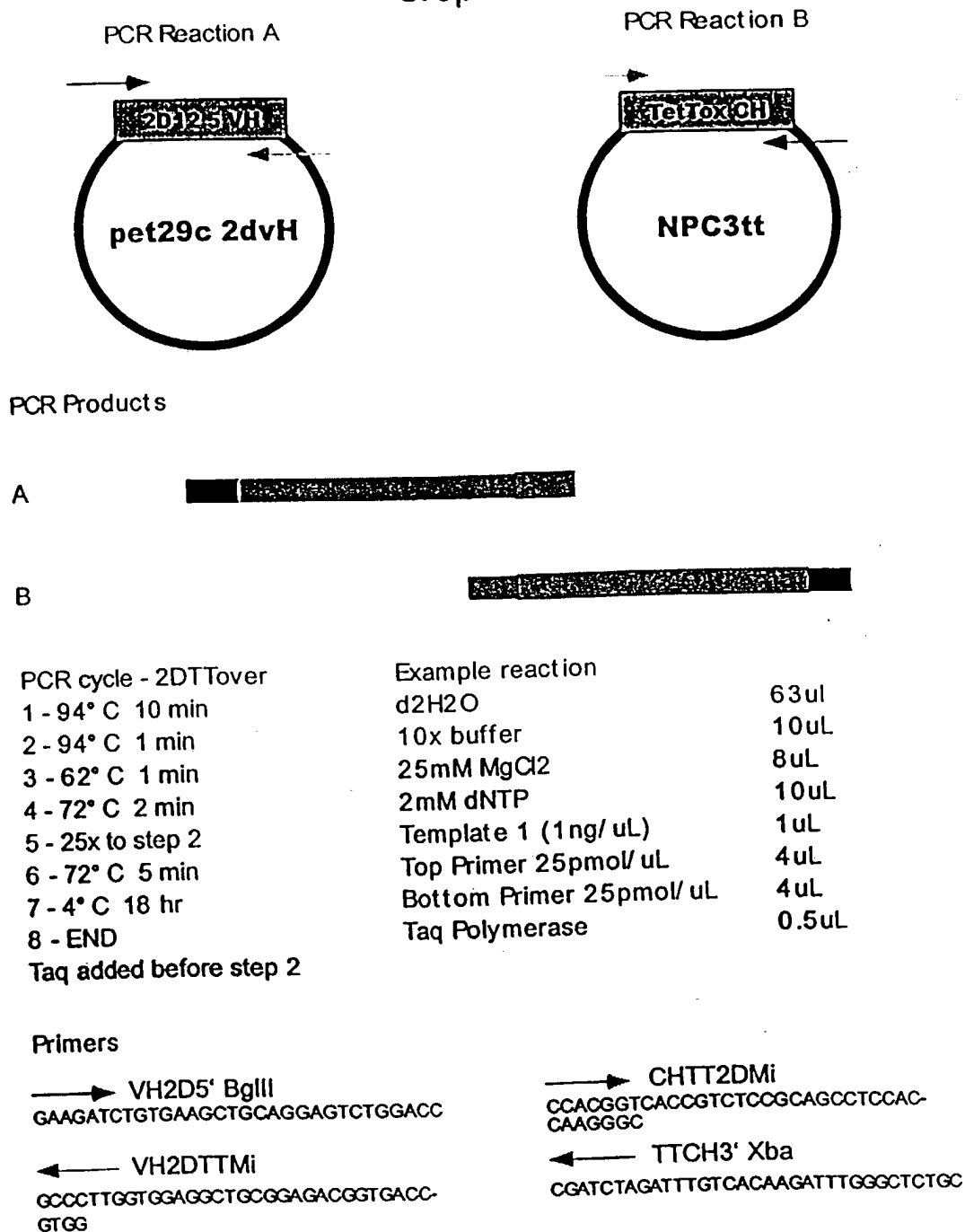
Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

2dVH-TTCH N87D_G53C_cloned	601	650
2dVH-TTCH N87D_G54C_clon d	601	650
2dVH-TTCH N87D_G55C_cloned	601	650
2dVH-TTCH expected sequence	601	650
2d12.5 VH native hybridoma			

		660	670	680
			
2dVH-TTCH_native cloned	651	AGCAGAGCCCAAATCTTGTGACAAATCTAGA	681	
2dVH-TTCH_N87D_cloned	651	681	
2dVH-TTCH_N87D_G53C_cloned	651	681	
2dVH-TTCH_N87D_G54C_cloned	651	681	
2dVH-TTCH_N87D_G55C_cloned	651	681	
2dVH-TTCH expected sequence	651	681	
2d12.5 VH native hybridoma				

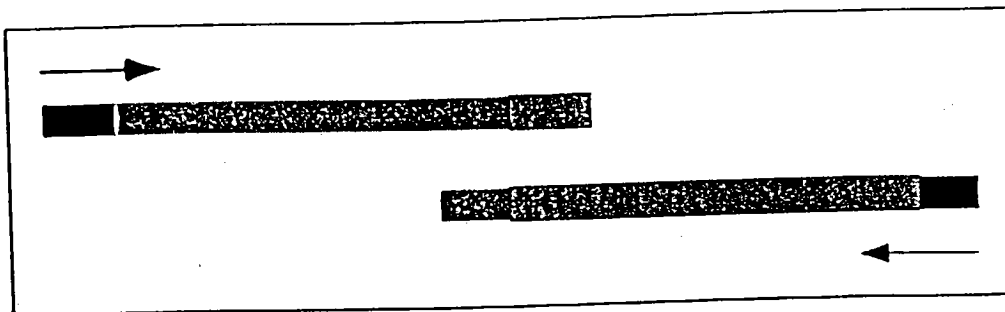
Strategy for Assembly of Chimeric 2D12.5 Heavy Chain

Step 1



Strategy for Assembly of Chimeric 2D12.5 Heavy Chain Step 2

PCR Reaction



PCR cycle - 2DTTVent

- 1 - 95°C 10 min
- 2 - 94°C 1 min
- 3 - 60°C 1 min
- 4 - 75°C 2 min
- 5 - 4x to step 2
- 6 - 94°C 1 min
- 7 - 63°C 1 min
- 8 - 75°C 2 min
- 9 - 25x to step 6
- 10 - 72°C 5 min
- 11 - 4°C 18 hr
- 12 - END

Vent added before step 2

Primers added before step 6

Primers

→ VH2D5' BglII
GAAGATCTGTGAAGCTGCAGGAGTCTGGACC

← TTCH3' Xba
CGATCTAGATTTGTCACAAGATTGGGCTCTGC

Example reaction

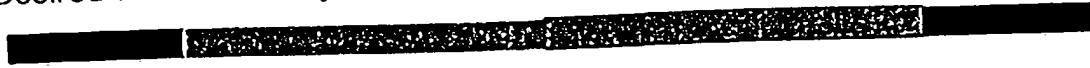
d2H2O	70ul
10x buffer	10uL
100mM MgSO4	0uL
2mM dNTP	10uL
Template 1(1ng/ uL)	1uL
Template 2(1ng/ uL)	1uL
Top Primer 25pmol/ uL	4uL
Bottom Primer 25pmol/ uL	4uL
Vent Polymerase	0.5uL

PCR Assembly Product



Strategy for Assembly of Chimeric 2D12.5 Heavy Chain Step 3

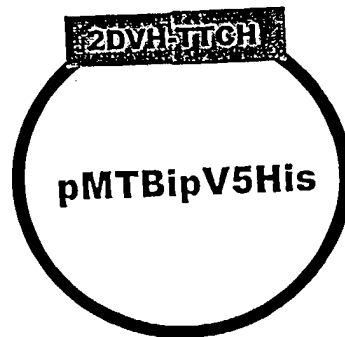
Desired PCR Assembly Product



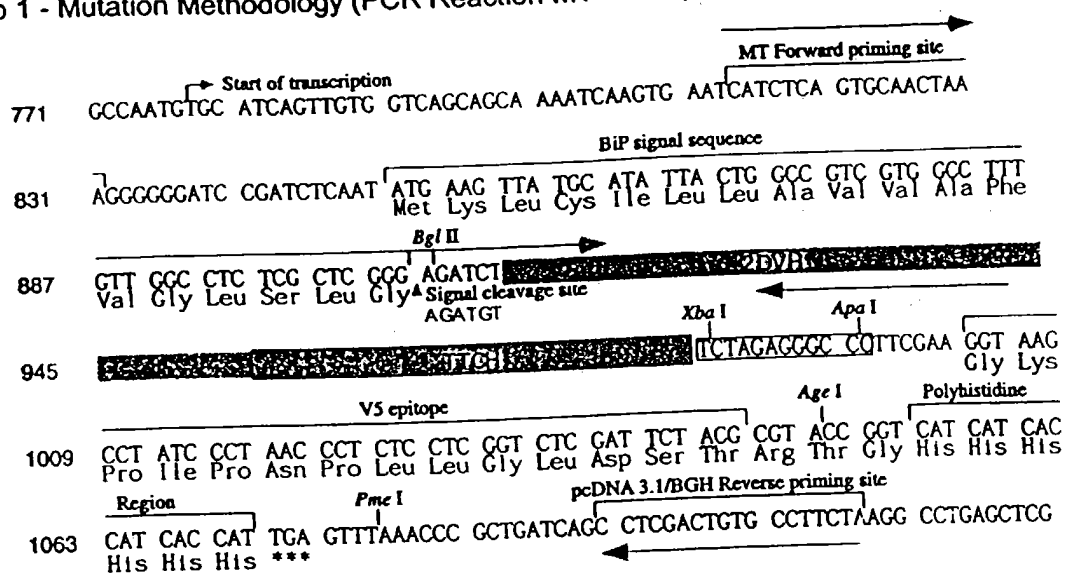
Restriction Digest PCR Product with Bgl II & Xba I



Ligate Restriction Digested PCR Product into pMTBipV5His
(S2 Cell Expression Vector, Propagated in XL-1 Blue E. Coli)



Step 1 - Mutation Methodology (PCR Reaction MT-VENT)



1st Set of PCR Reactions Producta A and B)



→
CATCTCAGTGCAACTAAA

MTforward

←
CATGGCTGTGTCATCAGCTTGCAGACTGTTC

2dvhN87D_pMTBip

OR

CGTGCTCCACAACCTCCATATCAC

G53C noncoding 2dG53c_pMT

OR

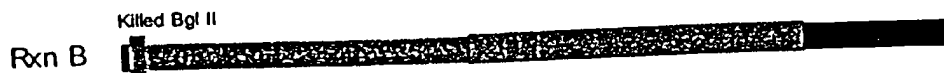
CCGTGCCACAACCACTCCATATC

G54C noncoding 2dG54c_pMT

OR

CCGTGCATCCACCACTCCATATC

G55C noncoding 2dG55c_pMT



→
GCTCGGGAGATGTGTGAAGCTG

2dvhKBgIII_pMTBip

←
TAGAAGGCACAGTCGAGG

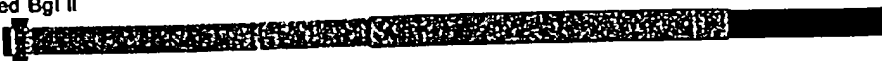
BGHreverse

Step 2 - Mutation Methodology (PCR Reaction VHMUTTAQ)

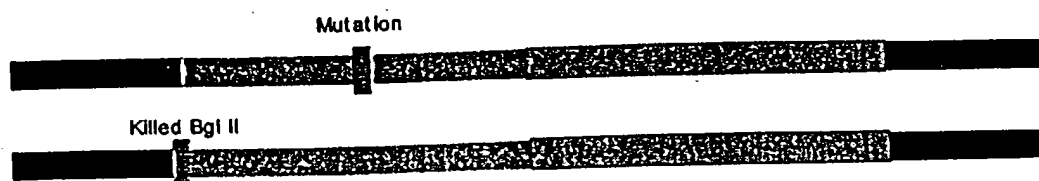
2nd PCR Reaction (Mix Products of reaction A and B)

- 1) Extend
- 2) Amplify with outer primers (MTforward and BGHreverse)

Rxn A 

Rxn B 

2nd PCR Reaction Products (Mixture - 2 Products of equal size)



Restriction Digest PCR Product Mixture with BglII and Xba1

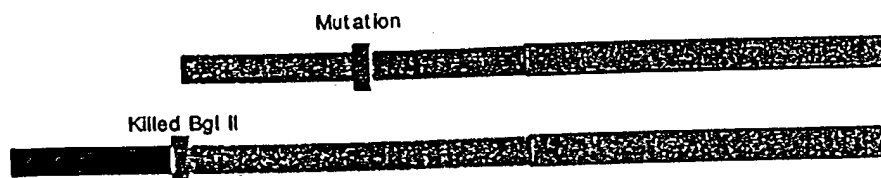


FIG. 10F

Step 1 - PCR Reaction MT-VENT

PCR cycle - MT-VENT

1 - 95° C 10 min
 2 - 94° C 1 min
 3 - 50° C 1 min
 4 - 75° C 2 min
 5 - 24x to step 2
 6 - 75° C 5 min
 7 - 4° C 18 hr
 8 - END
 VENT added before step 2
 Primers added before step 1

Example reaction

d2H2O	70ul
10x buffer	10uL
100mM MgSO4	0uL
2mM dNTP	10uL
Template (1 ng/uL)	1uL
Top Primer 25pmol/uL	4uL
Bottom Primer 25pmol/uL	4uL
Vent Polymerase	0.5uL

Step 2 - PCR Reaction VHMUTTAQ

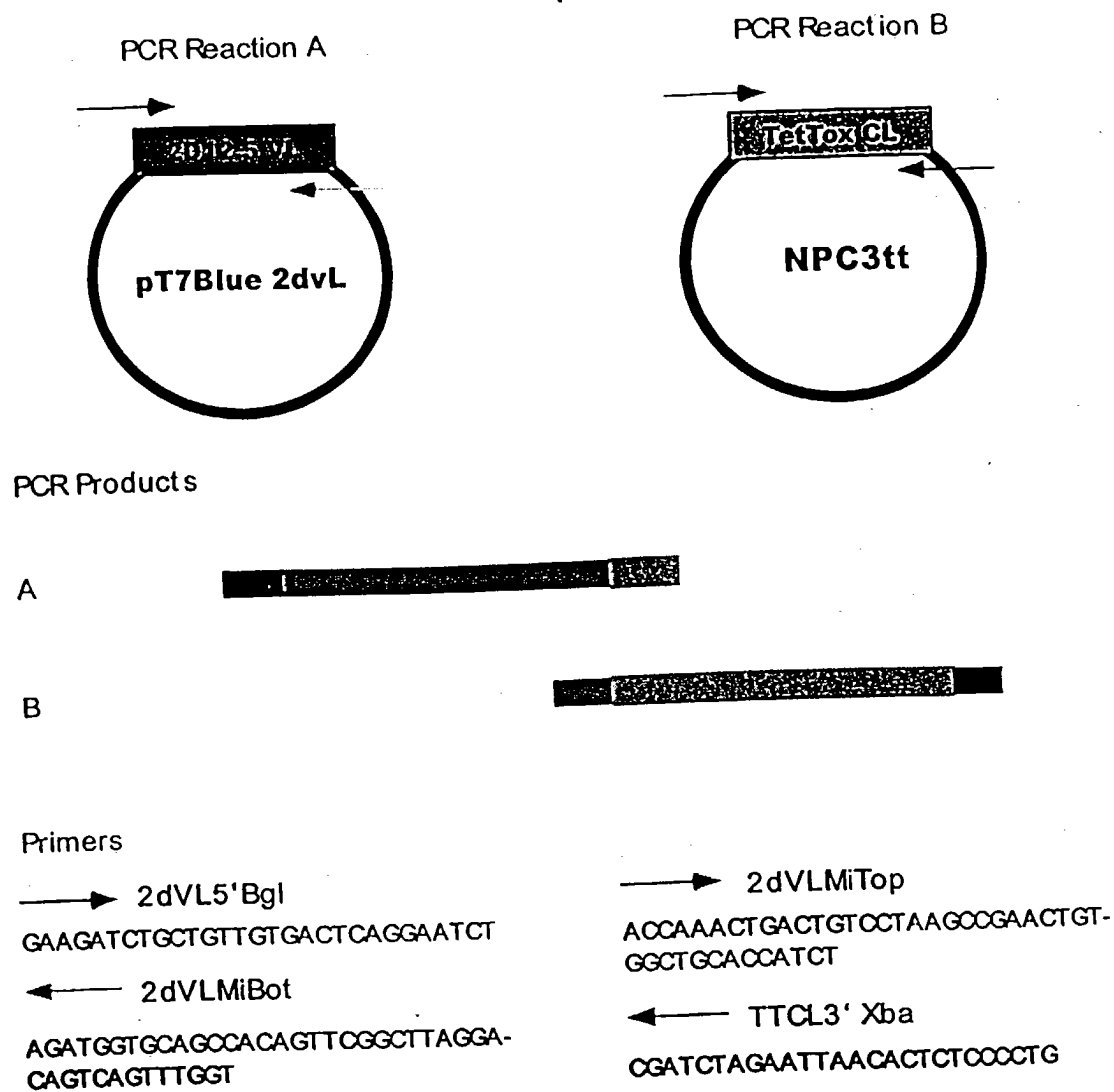
PCR cycle - VHMUTTAQ

1 - 95° C 10 min
 2 - 94° C 1 min
 3 - 68° C 1 min
 4 - 72° C 2 min
 5 - 4x to step 2
 6 - 94° C 1 min
 7 - 50° C 1 min
 8 - 72° C 2 min
 9 - 24x to step 6
 10 - 72° C 5 min
 11 - 4° C 18 hr
 12 - END
 Taq added before step 2
 Primers added before step 6

Example reaction

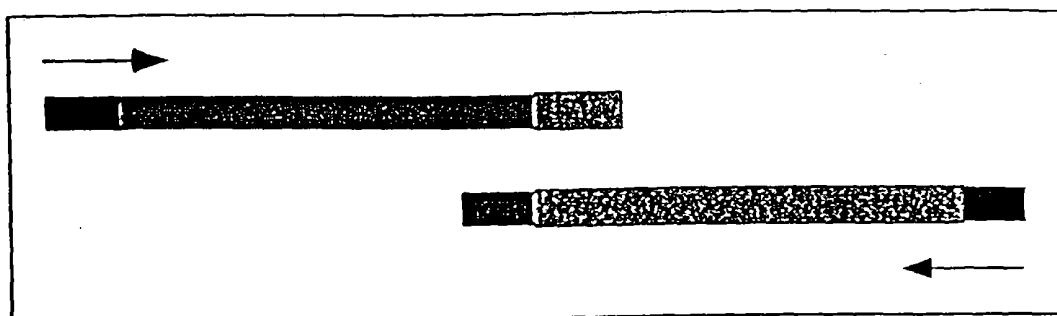
d2H2O	61ul
10x buffer	10uL
25mM MgCl2	8uL
2mM dNTP	10uL
Template 1 (1ng/uL)	1uL
Template 2 (1ng/uL)	1uL
Top Primer 25pmol/uL	4uL
Bottom Primer 25pmol/uL	4uL
Taq Polymerase	0.5uL

Strategy for Assembly of Chimeric 2D12.5 Light Chain Step 1



Strategy for Assembly of Chimeric 2D12.5 Light Chain Step 2

PCR Reaction



Primers

→ 2dVL5' Bgl

GAAGATCTGCTGTTGTGACTCAGGAATCT

← TTCL3' Xba

CGATCTAGAATTAACACTCTCCOCTG

PCR Assembly Product



Strategy for Assembly of Chimeric 2D12.5 Light Chain Step 3

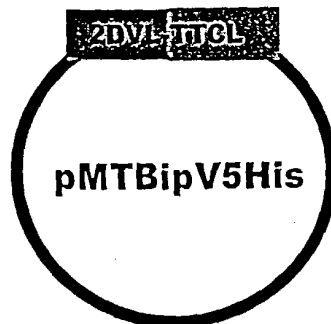
Desired PCR Assembly Product



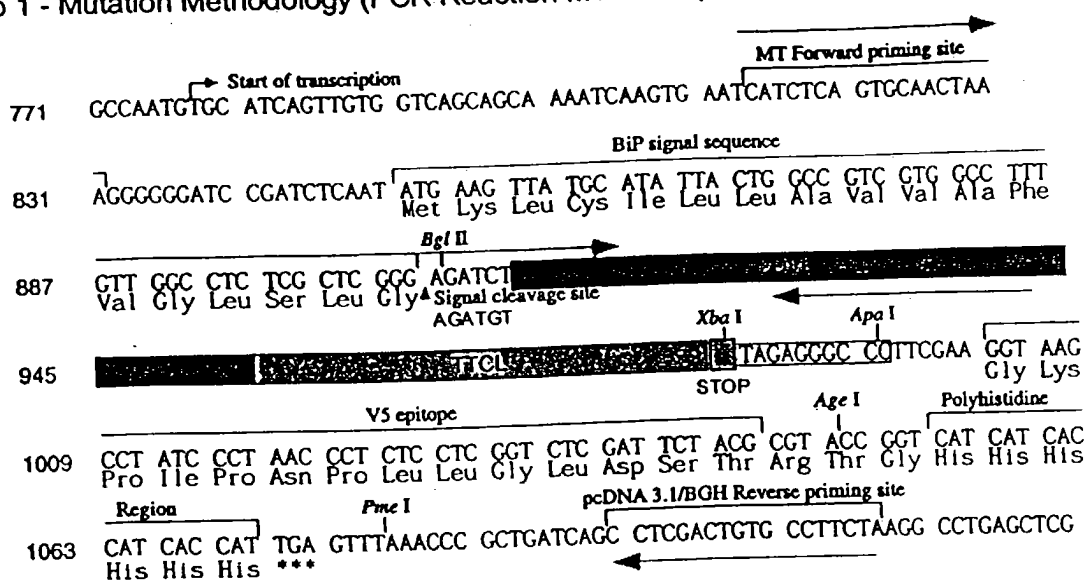
Restriction Digest PCR Product with Bgl II & Xba I



Ligate Restriction Digested PCR Product into pMTBipV5His
(S2 Cell Expression Vector, Propagated in XL-1 Blue E. Coli)



Step 1 - Mutation Methodology (PCR Reaction MT-VENT)



1st Set of PCR Reactions Producta A and B)



Step 2 - Mutation Methodology (PCR Reaction VHMUTTAQ)

2nd PCR Reaction Mix Products of reaction B)

1) Extend

2) Amplify with outer primers (MTforward and BGHreverse)

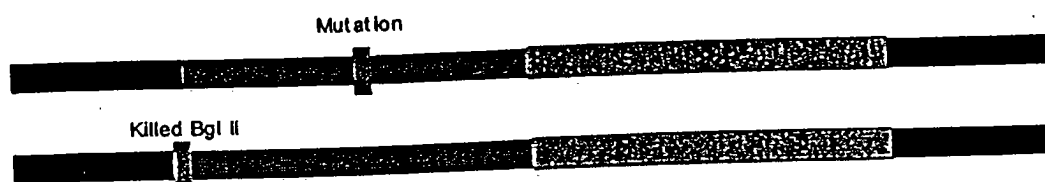
Rxn A

A horizontal black bar representing a DNA fragment. A small vertical tick mark is located near the right end. Above the bar, the word "Mutation" is written, with a vertical line pointing down to the tick mark.

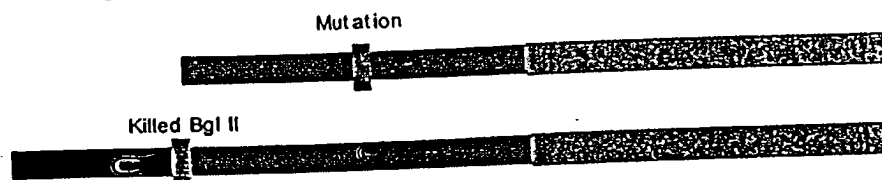
Rxn B

A horizontal black bar representing a DNA fragment. A small vertical tick mark is located near the left end. Above the bar, the text "Killed Bgl II" is written, with a vertical line pointing down to the tick mark.

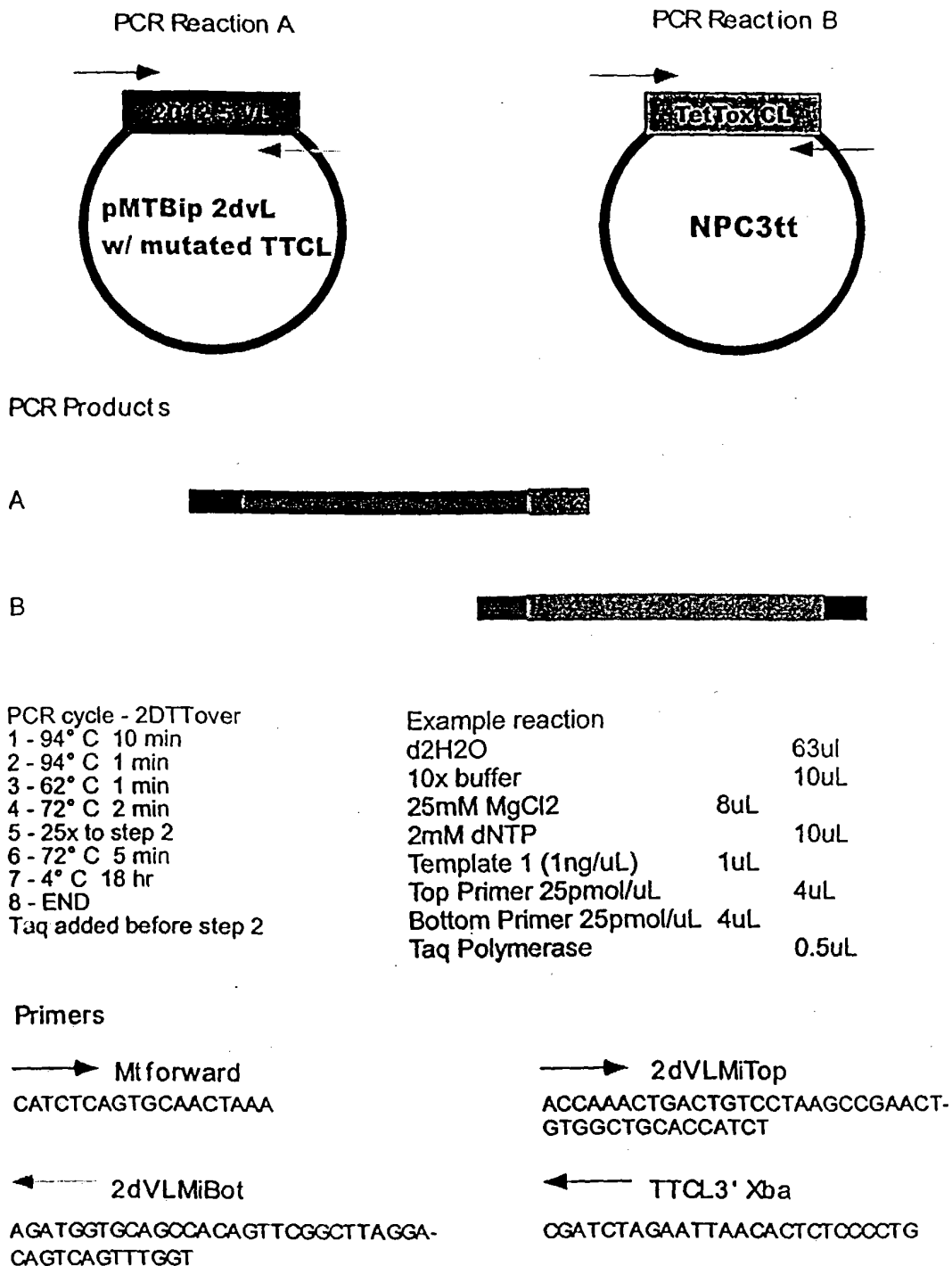
2nd PCR Reaction Products (Mixture - 2 Products of equal size)

Two horizontal black bars representing DNA fragments. The top bar has a small vertical tick mark near the right end, with the word "Mutation" written above it. The bottom bar has a small vertical tick mark near the left end, with the text "Killed Bgl II" written above it.

Restriction Digest PCR Product Mixture with BglII and Xba1

Two horizontal black bars representing DNA fragments. The top bar has a small vertical tick mark near the right end, with the word "Mutation" written above it. The bottom bar has a small vertical tick mark near the left end, with the text "Killed Bgl II" written above it.

Strategy for Assembly of Chimeric 2D12.5 Light Chain Step 4



Assembled Vectors for Transfection in S2 Cells

Each of the following has been cotransfected with the native light chain:

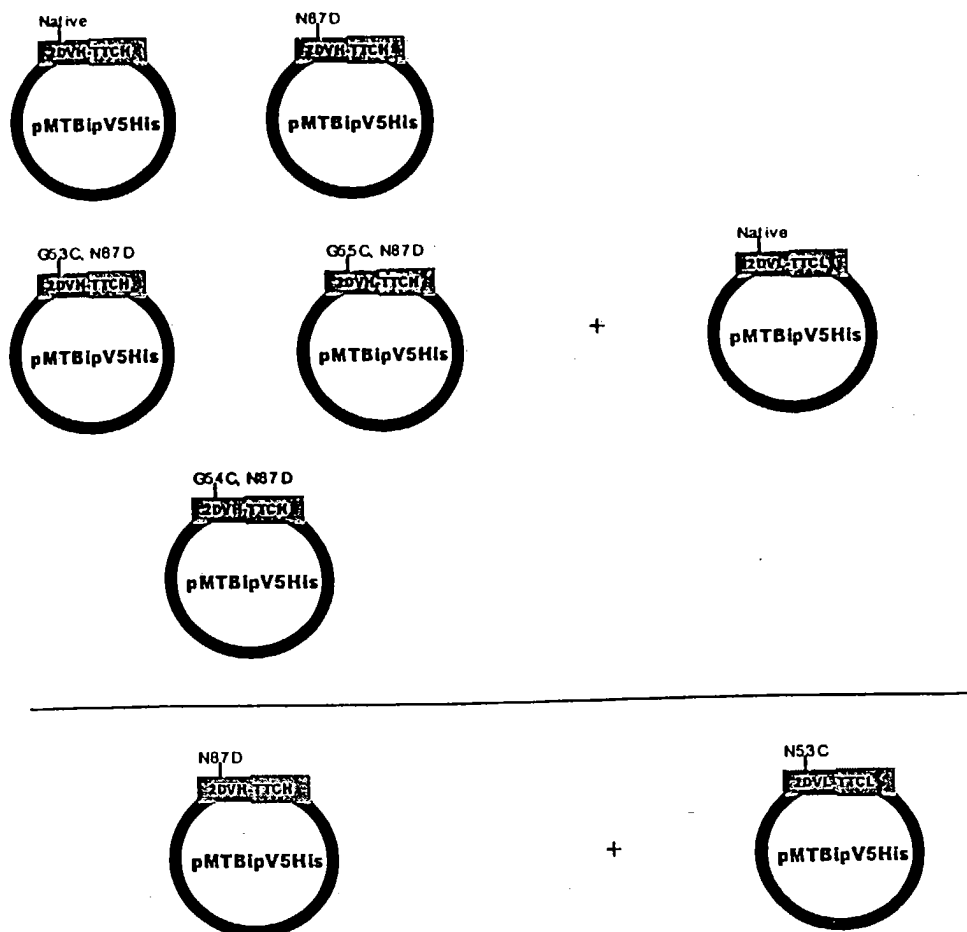


FIG. 12

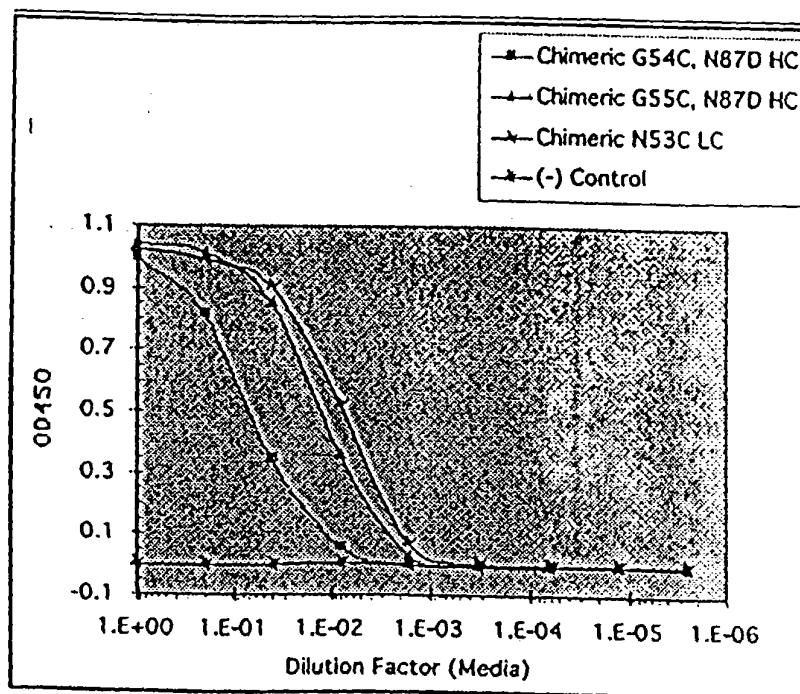
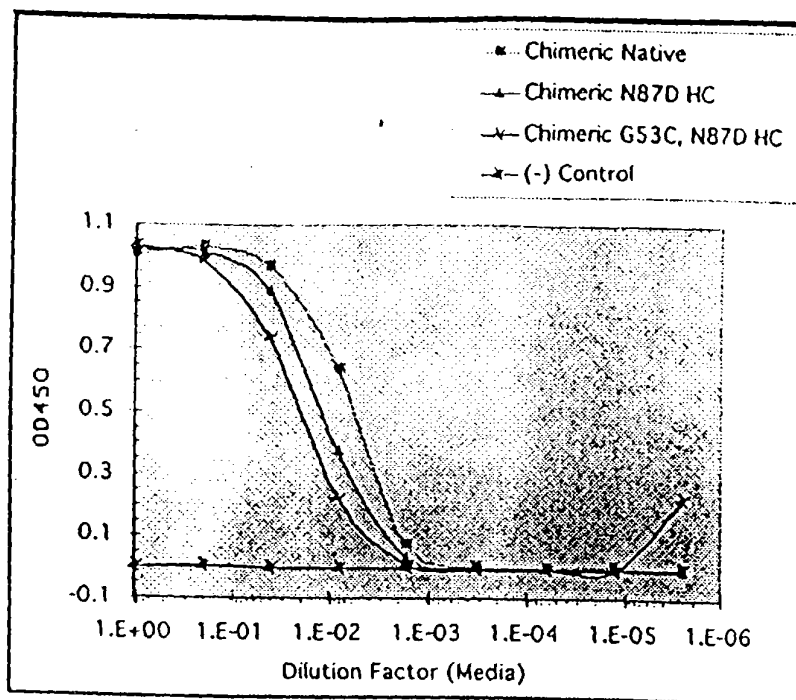


FIG. 13

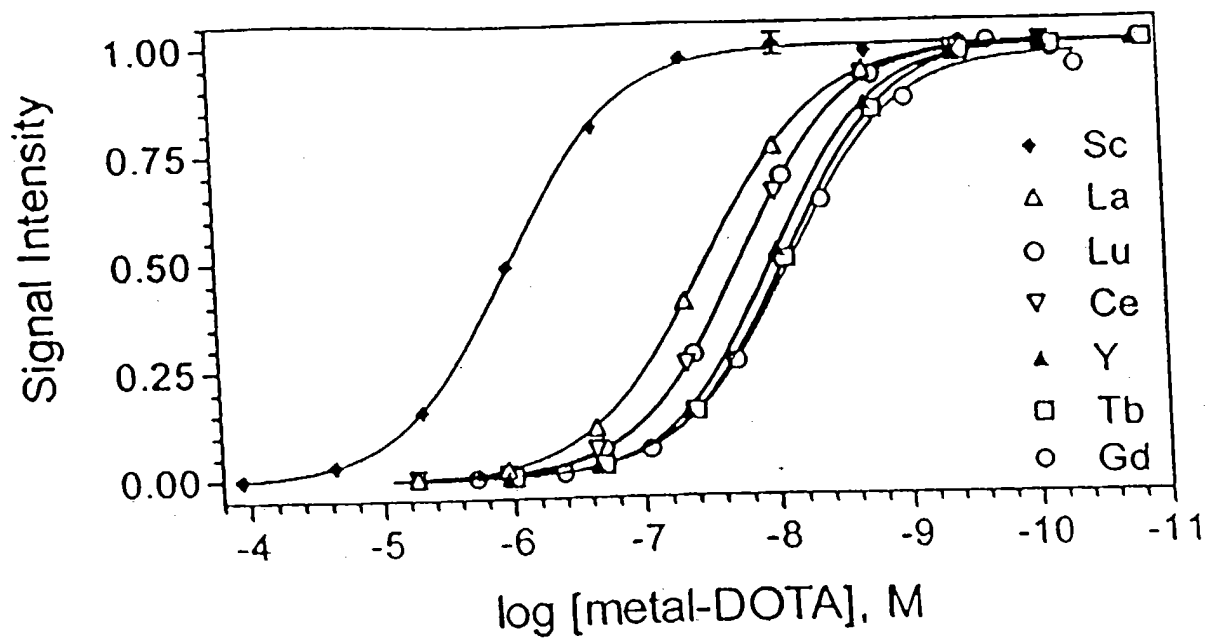


FIG. 14

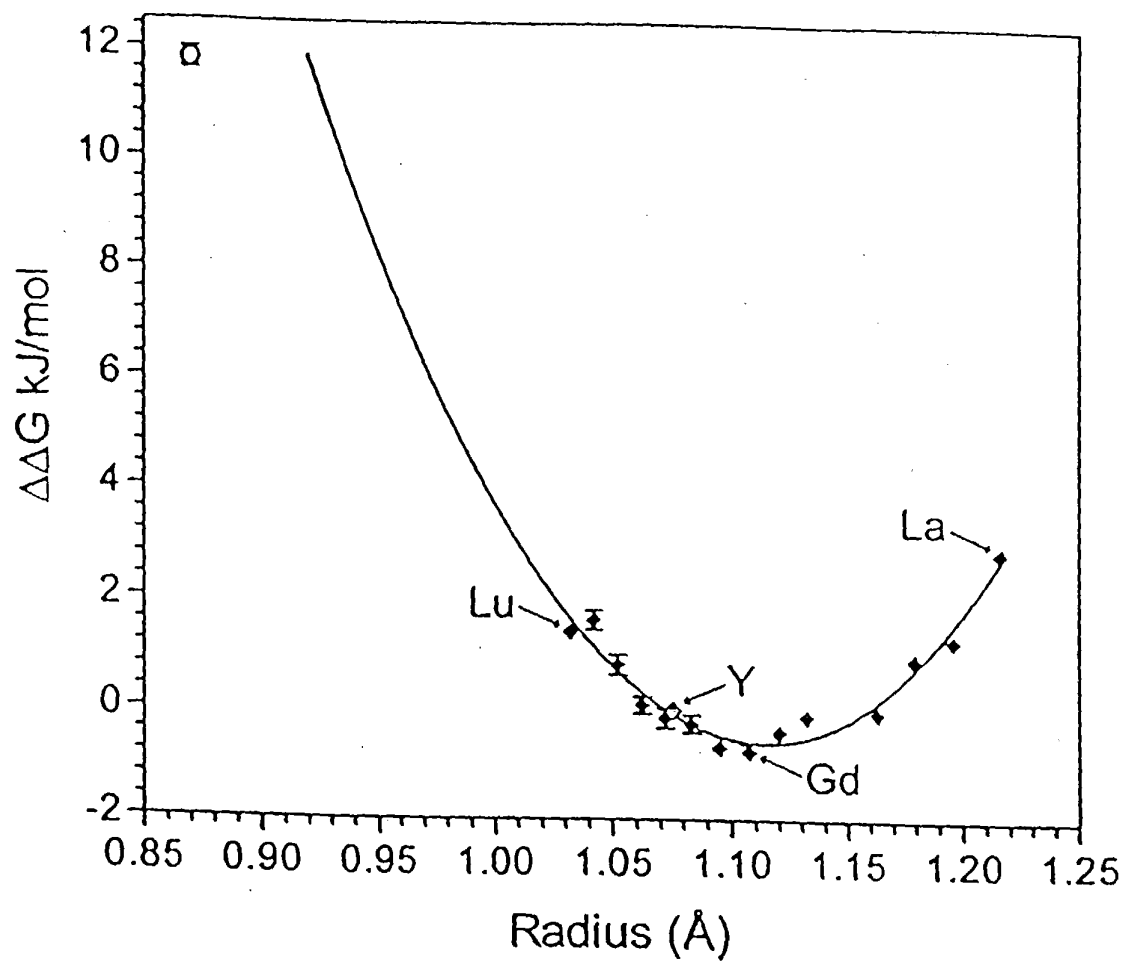


FIG. 15

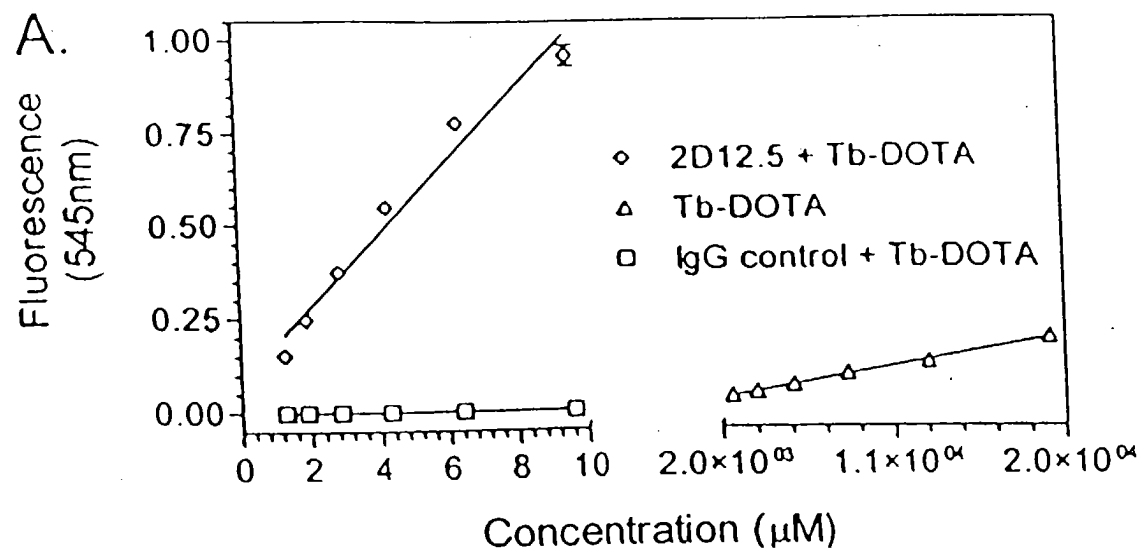


FIG. 16

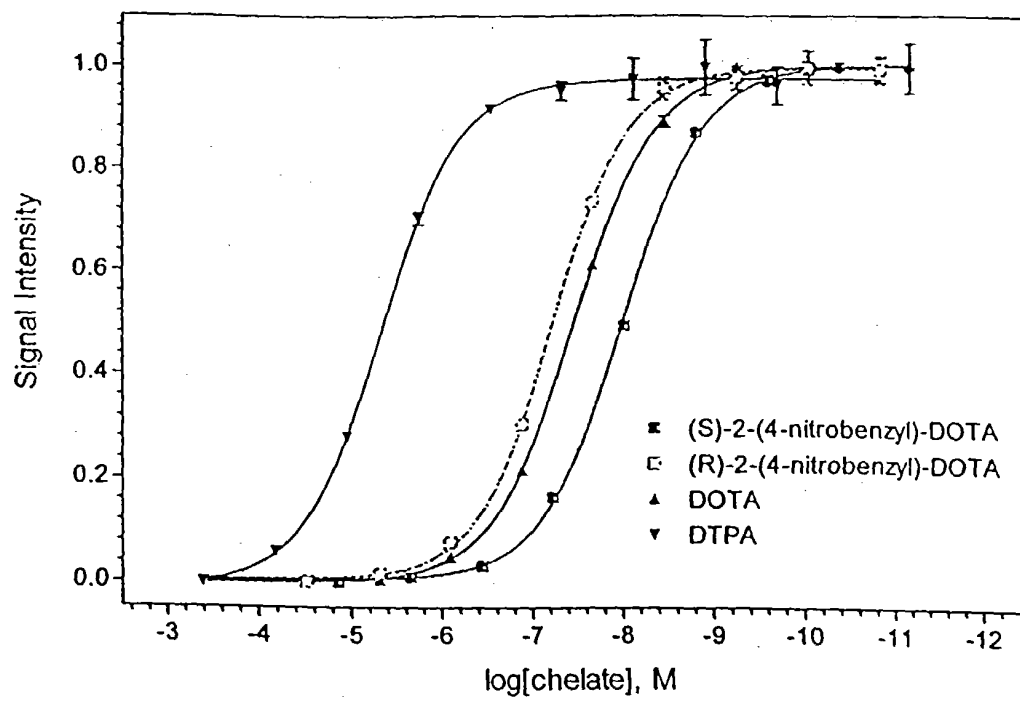


FIG. 17

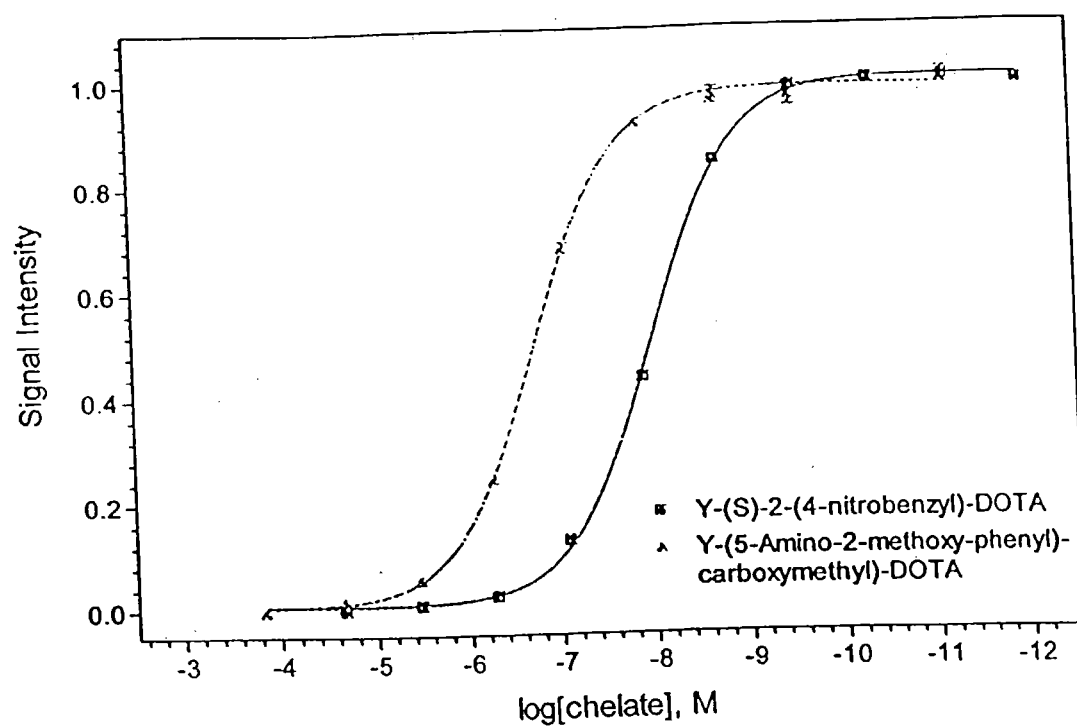


Figure 18

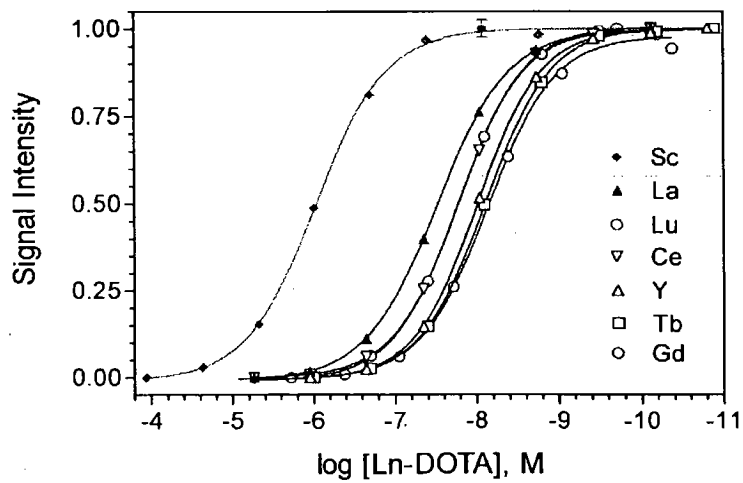
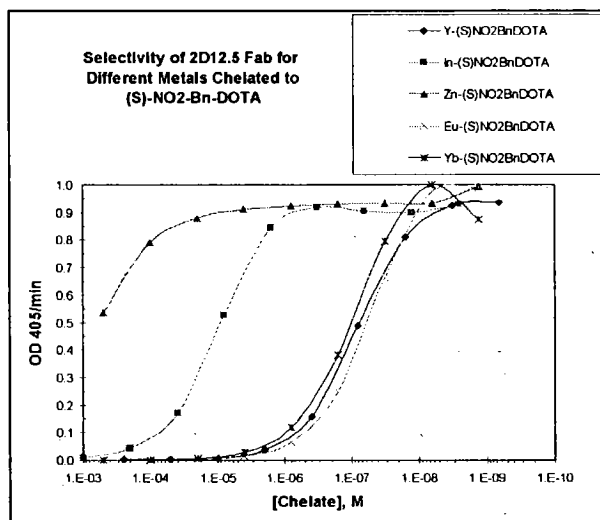
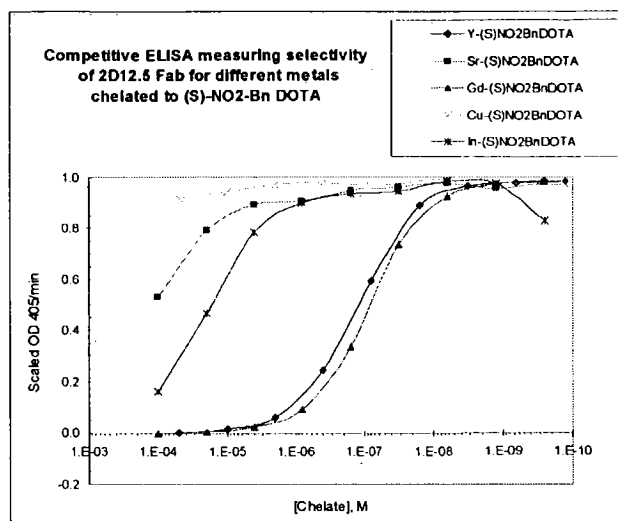


Figure 19

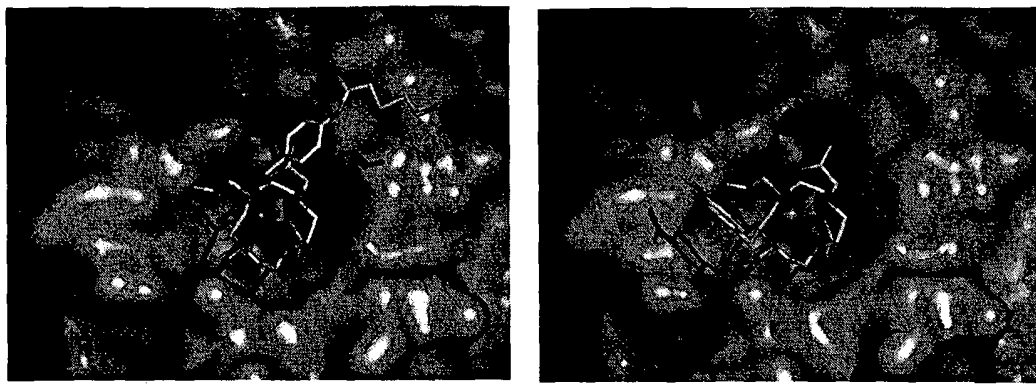
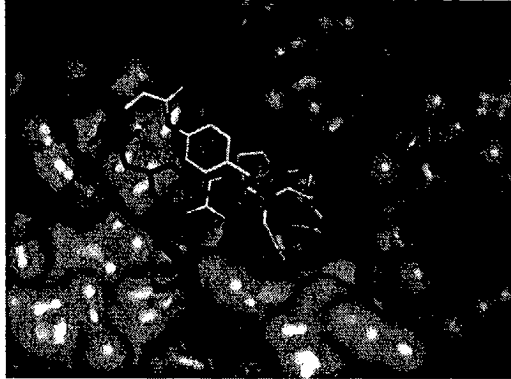


Figure 20

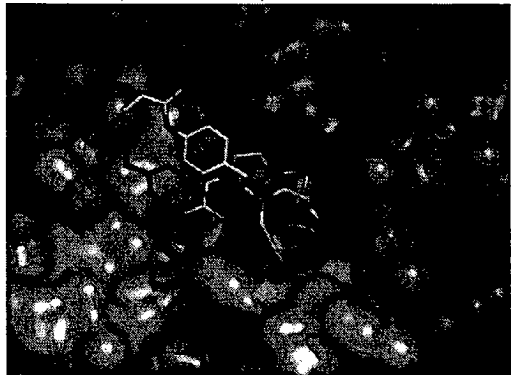
A. G54C, N85D HC, native LC



B. G55C, N85D HC, native LC



C. G56C, N85D HC, native LC



D. N85D HC, N53C LC

